INVESTIGATION OF ION-PEPTIDE INTERACTIONS USING A BIOCOMPATIBLE NANOPORE PROBE

A Thesis

by

SEAN BLOUNT BARD

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2012

Major Subject: Chemistry

INVESTIGATION OF ION-PEPTIDE INTERACTIONS USING A BIOCOMPATIBLE NANOPORE PROBE

A Thesis

by

SEAN BLOUNT BARD

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Approved by:

Chair of Committee,	Paul S. Cremer
Committee Members,	Manuel P. Soriaga
	Emile A. Schweikert
	Siegfried M. Musser
Head of Department,	David H. Russell

May 2012

Major Subject: Chemistry

ABSTRACT

Investigation of Ion-Peptide Interactions Using a Biocompatible Nanopore Probe.

May 2012

Sean Blount Bard, B.S. Rice University

Chair of Advisory Committee: Dr. Paul S. Cremer

The specific manner in which ions associate with a peptide surface is a subject of much research. The models currently proposed tend to rely either on computational results from overly simplified systems, or on observations of bulk solution behavior not applicable to peptide solvation. Herein we demonstrate a new platform for directly measuring specific ion interactions with peptides and use a pair of highly conserved model peptides to investigate specific mechanisms by which ions interact with a peptide surface.

A system for investigation of charge selective ion-peptide interactions using a conical glass nanopore was designed. The glass nanopore was coated using layer-by-layer depositions of poly(diallyldimethylammonium) chloride and sodium poly(styrenesulfonate) to control the size and charge selectivity of the nanopore. The tip of this nanopore probe was encapsulated in a 5% agarose gel to prevent peptide fouling. This probe was then used to measure the partitioning of cations to or from the surface of two model peptides: nonpolar V_5-120 and positively charged KV_6-112 elastin-like polypeptide (ELP). Partitioning was measured by clamping the current

through the pore at zero amps and measuring the resulting potential across the nanopore. This potential was used to determine the bulk concentration of electrolyte in a 1 mg/mL peptide in 0.1 M electrolyte solution.

Measurements were made with a patch clamp using chloride salts with the cations potassium, lithium, cesium, ammonium, and guanidinium at both room temperature and in an ice bath to ensure that the peptides were in their unfolded state and thus that all possible binding sites were exposed to the solution. All salts were observed to partition to the peptide surface with much less affinity than water, resulting in an increase in the bulk electrolyte concentration with the exception of ammonium, which showed a greater affinity than water for the KV_6-112 ELP in the ice bath measurements. These results demonstrate that cations do not favorably partition to nonpolar or cationic peptide surfaces.

TABLE OF CONTENTS

		Page
ABSTRACT……		iii
TABLE OF CONTENTS…….		v
LIST OF FIGURES……..		vi
LIST OF TABLES……		vii
CHAPTER		
I	INTRODUCTION……	1
	Objective……..	1
	Ion-Peptide Interactions……	1
	Elastin-like Polypeptides……..	4
	Conical Nanopores……...	5
	The Patch Clamp……..	7
II	MODIFYING THE GLASS NANOPORE PLATFORM.	9
	Introduction……..	9
	Experimental Procedures……	10
	Results and Discussion……	12
III	MEASURING ION-PEPTIDE INTERACTIONS……	14
	Introduction……..	14
	Experimental Procedures…….	15
	Results and Discussion……	16
IV	CONCLUSION……	22
REFERENCES…….		23
APPENDIX……..		26
VITA…….		31

LIST OF FIGURES

Page

Figure 1 Photograph of assembled probe…….. 11

Figure 2 I-V sweep of polyelectrolyte coated pore 35-07…….. 12

Figure 3 Calibration curve for cation selective probe 35-07
filled with 0.1 M KCl in ice bath…….. 17

Figure 4 1 mg/mL V_5-120 ELP in 0.1 M KCl in ice bath…….. 19

LIST OF TABLES

Page

Table 1 Results of room temperature measurements of pore potential in ELP solution……. 18

Table 2 Results of ice bath measurements of pore potential in ELP solution……. 20

CHAPTER I

INTRODUCTION

OBJECTIVE

The behavior of ions at peptide surfaces has been the subject of considerable research since 1888, when Franz Hofmeister realized that the addition of salts resulted in coagulation of the proteins in egg whites and that the extent of this effect depended on the nature of the salt used. Many scientists over the years have attempted to investigate the molecular origin of such behavior, but their research has been largely limited to the use of naturally occurring proteins, or computational simulations using simplified systems. The development of elastin-like polypeptides (ELPs) provides a library of easily modified peptides to examine these interactions in a platform more closely resembling actual proteins. Chapter II discusses an ion-selective nanopore probe and the steps to prepare it for salt measurements. Chapter III shows how the probe is used to analyze the interactions between a series of cations and two highly-conserved ELPs: one entirely nonpolar and one almost identical except for where one in every 35 residues has been changed from non-polar valine to cationic lysine.

ION-PEPTIDE INTERACTIONS

The Hofmeister series is a pattern of ions organized in order of their ability to denature proteins[1]. Salts that have the effect of solubilizing or "salting in" proteins are

---

This thesis follows in the style of *Journal of the American Chemical Society.*

called “chaotropes”, and those that stabilize protein structure are called “kosmotropes”. Many investigators have attempted to elucidate the specific mechanism of this phenomenon. In the 1940s and 50s, George Scatchard at MIT performed a series of studies on the physical chemistry of serum albumin (as it was a relatively easy to obtain protein and a popular subject of research in general at the time). He used newly developed ion exchange membranes to investigate the binding of various ions to human and bovine serum albumin[2,3]. Unfortunately, because of the limited access to purified proteins in the day, he was largely limited in his research to what few proteins were commonly available for research as modern genetic sequencing techniques had not yet been developed. As a result, research into specific mechanisms of ion binding was limited because of unsuitable materials. The existence of strength of ion binding sites could be determined, but not the specific nature thereof.

Ion hydration has been hypothesized to have a significant role in ion pair formation and therefore the structure and biological function of proteins. A recent view of the matter is shown in Kim Collins’ proposed empirical “Law of Matching Water Affinities” to describe how ions associate preferentially with each other depending on their relative affinity to each other versus their affinity to water[4,5]. In this model small, highly hydrated ions such as Li^+ and F^- (kosmotropes) will pair with each other because each is attracted to the other more strongly than to water, while large, less hydrated ions such as Cs^+ and I^- (chaotropes) will pair because water associates with itself more strongly than with either of the ions. This model was rationalized by the so-called “volcano plot” which showed a correlation between the enthalpies of solution of a

number of alkali halides and their absolute heats of hydration. In other words, ions that are strongly paired in solution exhibit endothermic solvation while those that are strongly paired exhibit exothermic dissolution. A similar relationship can be seen between the free energy of solution and the difference between the free energies of hydration for the species. While this empirical law applies most strongly to monoatomic, monovalent ions, it is relevant to biological systems because it suggests, as Collins states: "The major intracellular anions (phosphates and carboxylates) are kosmotropes, whereas the major intracellular monovalent cations (K^+; arg, his, and lys side chains) are chaotropes; together they form highly soluble, solvent-separated ion pairs that keep the contents of the cell in solution."[5]

Robinson and Harned's "localized hydrolysis" model represents another, earlier attempt at describing these phenomena[6]. This model describes small well-hydrated ions as associating strongly enough with the oxygen atom in water that a hydrogen atom partially dissociates from the water molecule and itself associates with an available nearby proton acceptor (typically the anion of the dissolved salt). This model indirectly also explains the "volcano" trend of ion association but does it from a perspective of ion interaction with water ions and the ability of an electrolyte to hydrolyze water. However, this explanation seems unsatisfactory from the perspective of explaining ion interactions with peptides as it doesn't explain the preferential partitioning of ions such as iodide towards the nonpolar portions of a peptide.

Jungwirth and associates have done a number of theoretical studies using model nanospheres with charged patches[7,8], individual amino acid residues[9-11], proteins[12], and

phospholipids[13]. These studies show that, in general, small, highly hydrated ions, such as lithium or fluoride should partition towards charged moieties of amino acids, while larger, weakly hydrated ions, such as cesium or iodide, should partition more towards the nonpolar backbone of the peptide.

ELASTIN-LIKE POLYPEPTIDES

Modern genetic sequencing techniques have allowed the facile synthesis of artificial peptides with specific sequences. ELPs are a family of engineered peptides that can be produced by recombinant DNA methods with well controlled composition and molecular weight and, due to their inverse phase transition, purified by relatively simple procedures[14,15]. Their sequence follows the pattern Val-Pro-Gly-Xaa-Gly where Xaa is a "guest residue" other than proline which can be changed through genetic modification. Because of this, a library of peptides can be engineered and expressed to allow the exploration of specific interactions in peptide systems without the limits inherent in investigating naturally occurring peptides and proteins. Previous research in this group has demonstrated the utility of a library of ELPs in analyzing the Hofmeister series and its effect on protein denaturation and structure[16-18].

The unique and easily exploitable themoresponsive reverse phase transition of ELPs has also made them a popular subject of research in the fields of bioengineering[19] and drug delivery[20-22]. ELPs tend to be easier to express and purify than many other proteins and peptides allowing the easy development of a library of peptides with specific modifications to the sequence. These advantages will allow the comparison of

the ion affinity of an ELP where every guest residue is valine (V_5-120) with a similar peptide where one in every seven valine guest residues is replaced with a lysine (KV_6-112). The number after the "-" refers to how many pentameric repeat units are in the peptide chain.

CONICAL NANOPORES

Asymmetric nanopores have been a vibrant field of study ever since the first demonstrated instance of a rectifying single nanochannel in 2001[23] because of their unique and versatile properties[24]. Nanopores can be made out of a variety of materials, such as polymers, glass/quartz, and silicon. Because of their small size, the dimensions of the nanopore are on a scale such that the thickness of the electric double-layer on their interior surface contributes significantly to their electronic properties[25]. Asymmetric nanopores have an asymmetric charge distribution along their axis which causes the pores to exhibit charge selectivity and a preferential direction of ion flow[26-28]. Nanopores can also be modified to change their properties and behavior through a variety of methods[29] such as self-assembled monolayer deposition[30], layer-by-layer polyelectrolyte deposition[31], or simply changing the nature of the electrolyte[32]. These properties will allow the construction of a biocompatible, charge selective electrode that will allow the investigation of ion association behavior of engineered model peptides.

Artificial nanopores have been proposed as a platform suitable for simulating biological nanopores in analyzing molecules and systems of biological relevance, because of their tunable chemical properties and greater robustness as compared to

systems involving biological nanopores and lipid bilayer membranes. As the name indicates, nanopores are pores with features measured in nanometers. At the nanoscale, the size of the pore is small enough that it becomes comparable to that of the electrical double layer. This results in an extremely high field strength at the narrow tip of the pore and the charge selectivity of the nanopore.

Because most artificial nanopores are made of materials with a negative surface charge, typically polymers that have carboxylate terminated chains or glass with a silanol terminated surface, the surface of the nanopore will attract a double-layer of cations. The preferential accumulation of positive charge at the pore tip creates a channel that preferentially allows the passage of cations through it. In addition to the observed charge selectivity, conical artificial nanopores also exhibit a preferential direction of current flow (ion current rectification) as a result of their geometric asymmetry. In other words, the magnitude of the current passing through the pore changes when the sign of the potential across the pore changes. As an example, when the anode is placed on the large side of the pore and the cathode is on the narrow side, a larger current magnitude is observed at negative potentials as cations flow from the narrow end to the wide end, while at positive potentials a lower current magnitude is observed as the cations flow in the less preferred large-to-narrow direction. The exact mechanism for this is still a matter of debate. However, Siwy's potential ratchet[27] describes the rectification as being a function of the potential energy distribution within the nanopore. On the other hand, Woermann describes the rectification as resulting from the difference of conductivity within the pore versus the bulk[26,28,33]. Finally, a model

proposed by Wei and Bard describes the diffuse double layer at the tip of the pore as creating a series of membranes of varying ionic permeability[34].

This preferential current flow exhibits itself not only when an electrical potential is placed across the pore, but also when the pore is placed under a concentration gradient.[35] When different concentrations of electrolyte are placed on each side of the nanopore, a nonzero zero-volt current can be observed, the magnitude of which depends on which side of the pore the higher concentration solution is placed. A higher magnitude of zero-volt current corresponds to greater charge selectivity as ions diffuse down the concentration gradient. This also means that if a potential is applied to hold the current through the pore at a fixed value (for our purposes zero), the magnitude of the potential necessary will exhibit a dependence on the direction of the concentration gradient in a similar manner with the magnitude of the necessary potential to clamp the current at zero. This correlates to the direction and magnitude of the concentration gradient. Thus, one could use a charge selective, conical nanopore system to measure the potential across a pore and correlate it with the bulk concentration of either the anion or cation species depending on pore construction. This will allow the determination of the extent of ion-peptide partitioning.

THE PATCH CLAMP

The patch clamp technique is a useful tool for analyzing not just biological pores, but also for investigating artificial nanopores. By using sensitive electronic amplifiers, a patch clamp can measure and resolve currents on the fractions of a picoamp scale.

This allows the measurement of small ion currents travelling through narrow pores or, similarly, the measurement of small potentials across a pore at a fixed current. This makes the patch clamp technique useful for investigating the behavior of the nanopore system described herein.

CHAPTER II

MODIFYING THE GLASS NANOPORE PLATFORM

INTRODUCTION

Conical nanopores are noteworthy for their rectifying behavior caused by their directional- and charge-selective properties. Glass nanopores from the White Group at the University of Utah were used because of their robust nature and convenient form-factor. Since the hand polished glass nanopore electrodes are, on average, larger than their track-etched counterparts, additional preparations were needed to bring the nanopore to a size more suitable for the measurements while simultaneously preserving the unique surface charge characteristics of the nanopore. Layer-by-layer polyelectrolyte depositions are known as a method for changing the size and charge selectivity of a nanopore[31,36]. The strong polyelectrolytes poly(diallyldimethylammonium) chloride (PDDA) (a cationic polyelectrolyte) and sodium poly(styrenesulfonate) (PSS) (an anionic polyelectrolyte) were chosen so as to keep the charge state of the pore consistent throughout the measurements.

As polyelectrolyte films are highly susceptible to protein adsorption regardless of the charge of the protein[37], it is necessary to protect the surface from being fouled by the peptides analyzed. Agarose is well known to form a biocompatible porous gel. The size of the pores can be controlled by changing the percentage of agarose in the gel and the setting conditions[38]. Additionally, at low salt concentrations, agarose is resistant to peptide and protein adsorption, thus its regular use in salt-gradient chromatography for

protein purification[39]. Because of this, it was chosen as a protective layer to prevent protein fouling of the polyelectrolyte surface of the probe.

EXPERIMENTAL PROCEDURES

Materials. Nanopore electrodes made from Corning borosilicate glass were obtained from the White Group at the University of Utah[40]. The pores were sized by the White Group before shipping. All electronic measurements were made with an Axon AxoPatch 200B patch-clamp amplifier and Digidata 1322 digitizer using pClamp and ClampFit software (Molecular Devices, Sunnyvale, CA). The water used was in all experiments was obtained from a Barnstead Nanopure (Barnstead, Dubuque, IA) water system which was purified to 18 MΩ/cm. Potassium chloride, poly(diallyldimethylammonium) chloride, sodium poly(styrenesulfonate), and agarose were obtained from Sigma-Aldrich.

Nanopore modification and characterization. Probe 35-07 was filled with 0.1 M potassium chloride by use of a plastic capillary pipette and a silver/silver chloride electrode was inserted. The glass nanopore electrode was immersed alternately in solutions of PDDA and PSS. The current-voltage (I-V) response of the pore was measured after each deposition to monitor the progress of the process. I-V measurements were taken by sweeping the voltage from -1 V to +1 V and back over the course of approximately 23 seconds in 0.1 M KCl. Three sweeps were taken and the results averaged. For this set of experiments, the probe was left with a negatively charged (cation selective) polyelectrolyte outer surface.

After the polyelectrolyte deposition, a 5% agarose gel was gently heated on a hot plate in 0.1 M KCl until thoroughly mixed and melted. The filled probe was dipped in the molten gel and then immersed in a 0.1 M potassium chloride solution to let it set and cool. This probe was used to perform all measurements. A photograph of the encapsulated and assembled probe is shown in Figure 1:

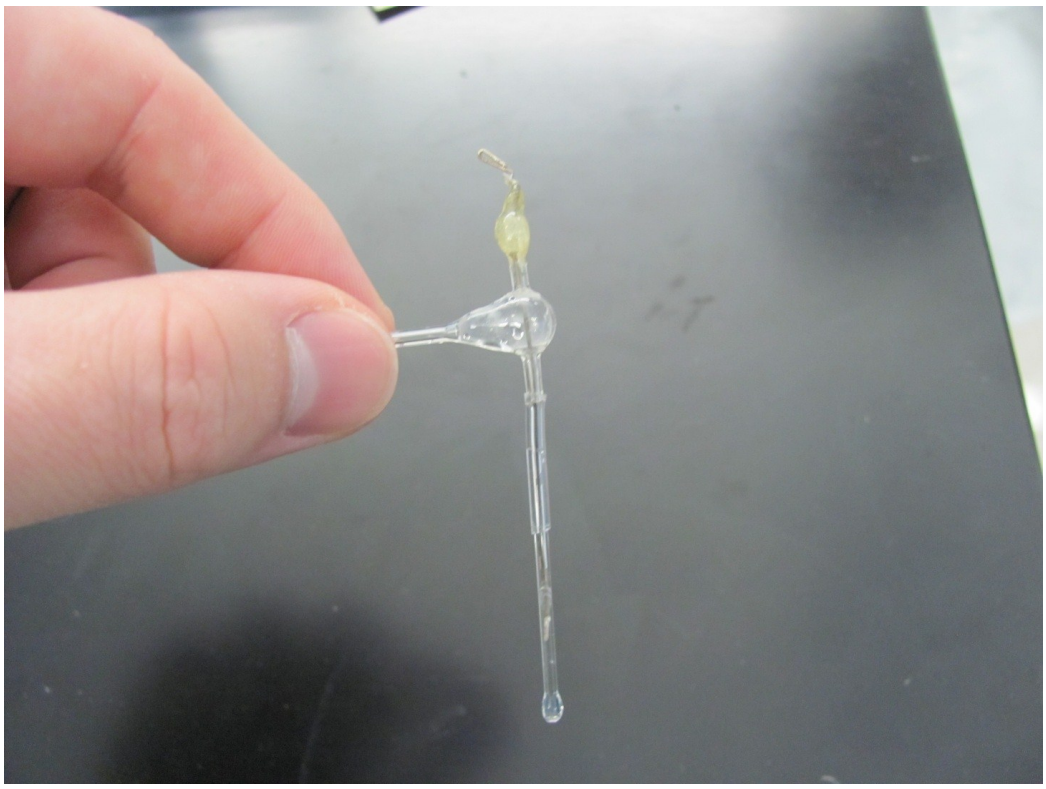


Figure 1. Photograph of assembled probe.

RESULTS AND DISCUSSION

The I-V sweep of the coated and encapsulated pore shown in Figure 2 demonstrates the rectifying behavior of the pore after five alternating depositions of PDDA and PSS:

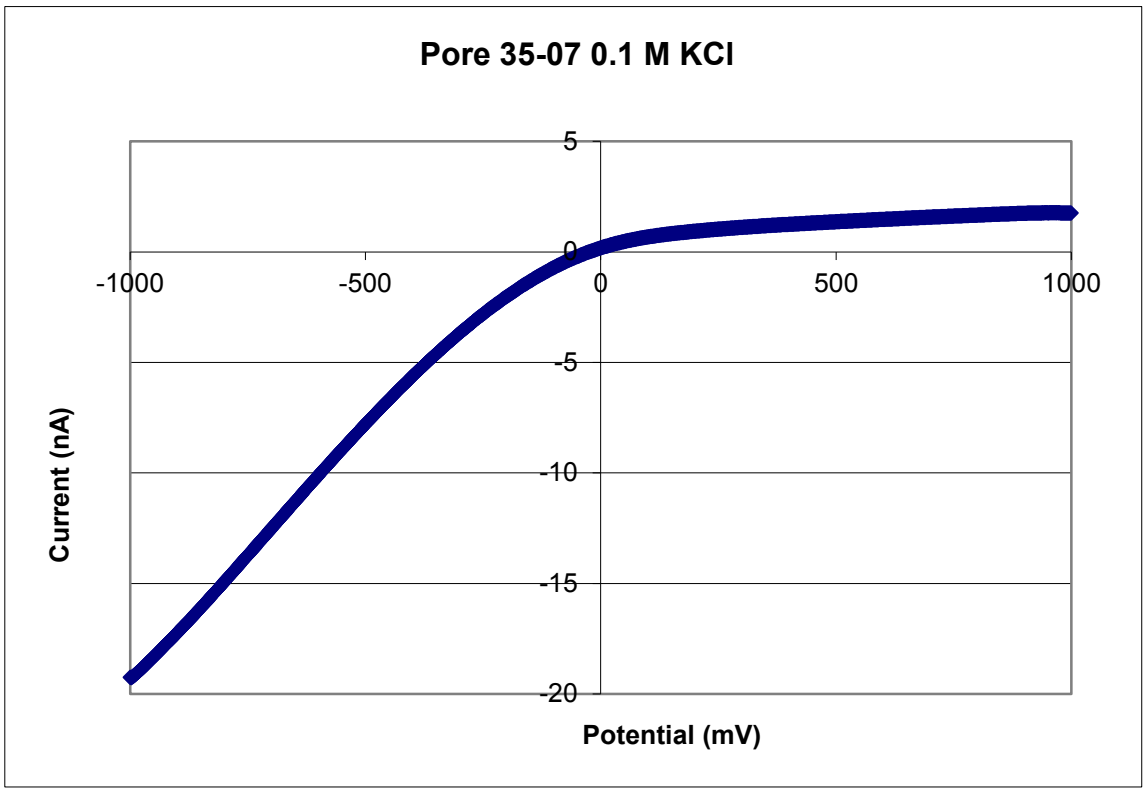


Figure 2. I-V sweep of polyelectrolyte coated pore 35-07.

It can be seen that the pore exhibits cation-selective behavior with the preferred direction of travel going from the outside of the probe to the inside as the magnitude of

the current travelling through the pore is much greater when the pore is held at a negative potential and much smaller when the pore is held at a positive one.

CHAPTER III

MEASURING ION-PEPTIDE INTERACTIONS

INTRODUCTION

When a protein or peptide is dissolved in a salt solution, molecules of the solvent associate with the surface of the peptide, thus solvating it. Along with the solvent, a certain portion of the ions in solution will partition to the surface of the peptide. Because of this, the quantity of the ions in bulk solution will decrease. This causes a change in the conductivity of the solution as a portion of the ionic species will have partitioned out of the solution, thus changing the concentration of the bulk phase. The extent of this partitioning is a function of the affinity of the ion to the peptide relative to that of water. As different amino acids have different ion affinities, different ELPs will have different ion affinities depending on the nature of the guest residues. If the ELP exhibits a greater affinity for water than for the ions in question, then more water will be partitioned away from the bulk solution compared to the ionic species. Thus, the bulk concentration of the solution will appear to increase. Alternatively, if the ions have a greater affinity for the peptide in question than water does, then more ions will partition out of the bulk relative to water and the electrolyte concentration of the bulk solution will decrease. For example, a positively charged ELP like KV_6-112 would be expected to bind anions more strongly than cations. A charge selective probe of the type proposed here will be able to investigate these interactions and separate anion-specific interactions from cation-specific ones.

EXPERIMENTAL PROCEDURES

Materials. Peptide solutions were made at a concentration of 1 mg/mL from lyophilized peptide aliquots. Peptides were prepared from plasmids obtained from the Chilkoti laboratory at Duke University, expressed in *E.Coli* BLR(DE3) competent cells according to standard procedure and purified using the inverse thermal cycling method described in previous research.[17] Purity was confirmed by comparing lower critical solution temperature (LCST) to a reference value supplied by the Chilkoti lab. Concentration was determined by UV-Visible spectroscopy at 280nm with an Agilent 8453 spectrometer (Agilent Technologies, Santa Clara, CA). Electrolytes tested were potassium chloride (Sigma-Aldrich), lithium chloride (Acros), cesium chloride (MP Biomedical), ammonium chloride (MP Biomedical), and guanidinium chloride (EM) at original concentrations of 0.1 M.

Calibration of the nanopore probe. Calibration measurements were performed by immersing the agarose-encapsulated probe in various electrolyte solutions and running current-clamp measurements at I = 0 until the potential was observed to have stabilized. Calibration curves to allow the determination of the bulk concentration of electrolyte were measured by taking aliquots of the electrolyte in question in concentrations of 0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, and 1 M and measuring the resulting potential at a I = 0. Measurements were done once for each aliquot.

Measuring ELP-ion interactions. Measurements with both V_5-120 and KV_6-112 ELPs were made at concentrations of 1 mg/mL (approx. 5.0×10^{-5} M) in 0.1 M solution of electrolyte. Measurements were taken by observing the potential required to

hold the current through the pore at zero. A baseline was established by immersing the probe in a solution of 0.1 M electrolyte until the potential was observed to have stabilized. The probe was then moved to a solution of the relevant ELP diluted to 1 mg/mL with the same electrolyte. The potential was measured until it was observed to have stabilized for several minutes. The value was found on the appropriate calibration curve to determine the bulk value of the electrolyte concentration in the ELP solution. As the phase transition temperature of V_5-120 is very close to room temperature[17], measurements were also performed in an ice bath to ensure that the peptides were in their fully unfolded states during measurement. Measurements were performed once each at room temperature and in an ice bath with each ELP in each salt.

RESULTS AND DISCUSSION

A representative sample of a calibration curve is shown in Figure 3 (other calibration curves can be found in the appendix). A logarithmic fit curve was generated, which allowed unknown solution concentrations to be determined. It can be seen that the greatest change in potential occurs in the region where the outside concentration is significantly lower than the electrolyte concentration inside the probe, and thus in retrospect, more accurate results would be obtained by using a higher concentration of electrolyte (1 M or greater) inside the pore to amplify charge selectivity[35].

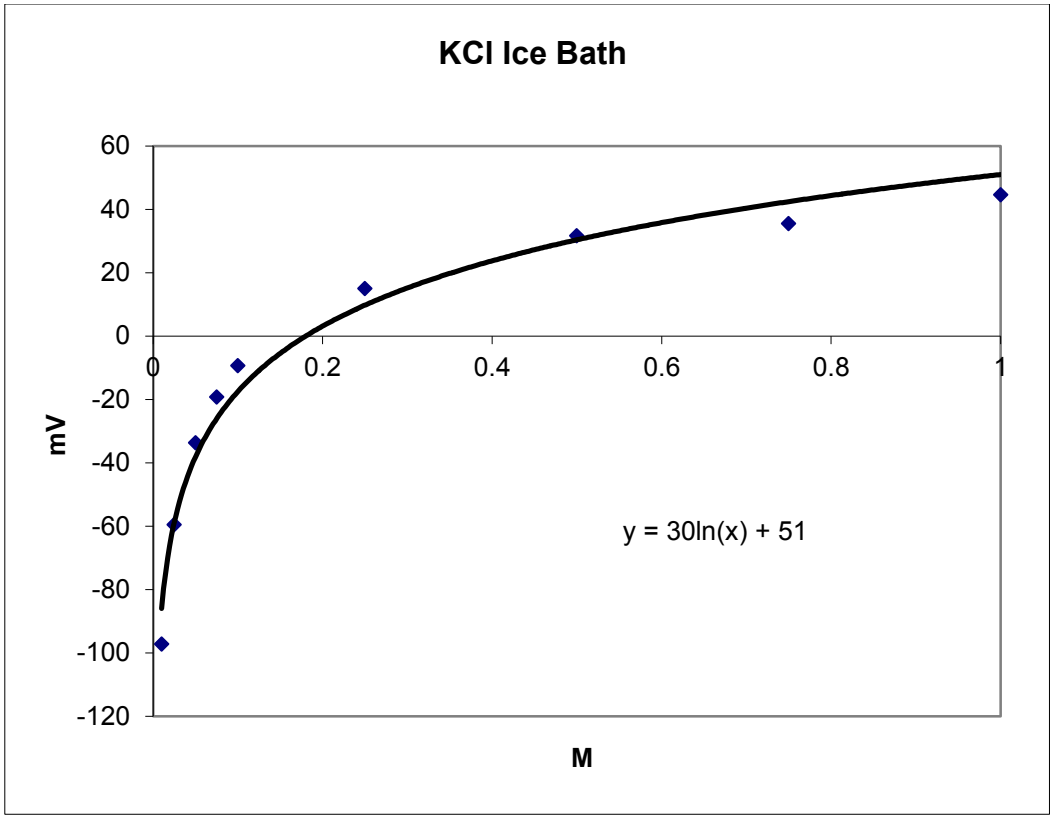


Figure 3. Calibration curve for cation selective probe 35-07 filled with 0.1 M KCl in ice bath. M refers to the bulk concentration of the sample analyzed.

In Table 1 are shown the data from room temperature measurements. Results from ice bath measurements are shown in Table 2. The columns with results expressed in mV refer to the potential measured when the probe was immersed in a 1 mg/mL peptide solution dissolved in 0.1 M chloride solution with the respective counterion. The potential measurement was determined by taking the average value of the "With ELP" portion of the readout after the potential had been observed to have stabilized. A representative example of the readout is show in Figure 4. The bulk concentration results were determined by examining the relevant calibration curve and finding the respective bulk concentration value for the measured potential on the fit curve.

Table 1. Results of room temperature measurements of pore potential in ELP solution. All measurements made with 0.1 M electrolyte in 1 mg/mL ELP solution.

Cation	V_5-120 Potential Measurements (mV)	Bulk Conc. (M)	KV_6-112 Potential Measurements (mV)	Bulk Conc. (M)
NH_4^+	-23	.15	-24	.15
Cs^+	-29	.14	-28	.15
K^+	-24	.13	-28	.12
Li^+	-49	.12	-49	.13
Gdn^+	-21	.18	-23	.17

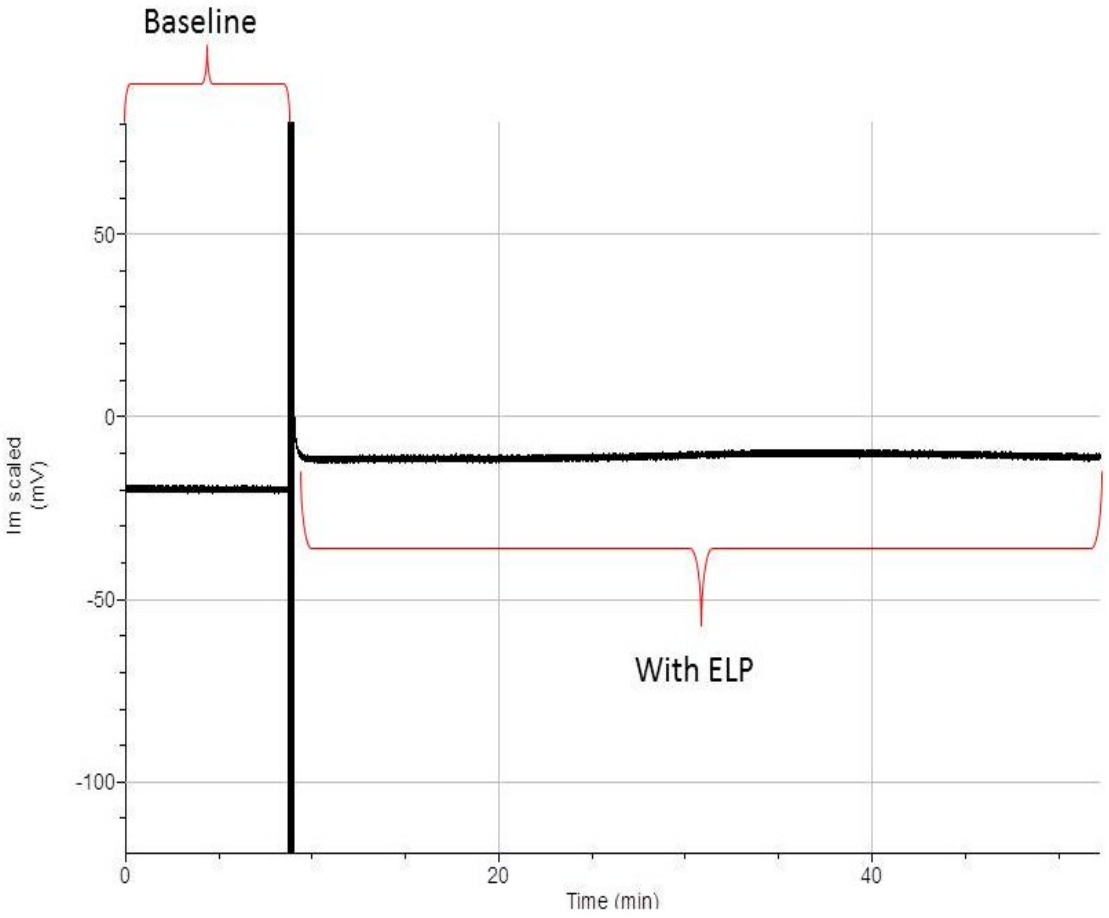


Figure 4. 1 mg/mL V_5-120 ELP in 0.1 M KCl in ice bath.

Table 2. Results of ice bath measurements of pore potential in ELP solution. All measurements made with 0.1 M electrolyte in 1 mg/mL ELP solution.

Cation	V_5-120 Potential Measurements (mV)	Bulk Conc. (M)	KV_6-112 Potential Measurements (mV)	Bulk Conc. (M)
NH_4^+	-17	.11	-33	.05
Cs^+	-7.4	.19	-11	.16
K^+	-10	.12	-13	.11
Li^+	-38	.11	-37	.12
Gdn^+	-10	.22	-9.5	.23

It can be seen that in each case, the cation in question associates much less preferentially with the V_5-120 ELP. For the alkali cations, the trend appears to follow the cation Hofmeister series although ammonium and guanidinium do not follow this pattern. The measured potentials of the solutions with V_5-120 ELP and all ions except ammonium with KV_6-112 measured above the baseline for a 0.1 M solution, indicating an increase in the bulk concentration of the cation in solution, or in other words, a greater preference for water instead of salt at the protein surface. The results from the room temperature V_5-120 measurements show a greater divergence from the ice bath measurements. The alkali salt measurements for KV_6-112 are very close to the ice bath measurements though ammonium and guanidinium did not follow this pattern.

Interestingly, these data seem to conflict with the model proposed by the Law of Matching Water affinities, which predicts that weakly hydrated Cs^+ should associate with the surface of the entirely nonpolar V_5-120 to a greater extent (or at least, partitioned away less strongly) than highly hydrated Li^+. It is also somewhat counterintuitive that the positively charged KV_6-112 appears to repel cations less strongly than the uncharged V_5-120. They do, however, confirm the claim that cations only associate with charged and polar sidechains of peptides[11]. This may be part of the reason why the cation Hofmeister series is much weaker than the anion one.

CHAPTER IV

CONCLUSION

In summary, it has been demonstrated that a conical, glass nanopore probe can be used as a platform for constructing a biocompatible probe for investigating ion-peptide interactions and that ELPs can be used in conjunction with this probe to investigate interactions between ions and different residues in these engineered peptides. Using this platform and a library of ELPs, such as highly conserved sequences like V_6F-28 and QV_6-120, it will be possible to investigate specific interactions involved in protein solvation and quantify the strength of these interactions and evaluate the validity of computational simulations. This platform could also be easily adapted to investigate anion-peptide interactions.

REFERENCES

(1) Hofmeister, F. *Arch. Exp. Pathol. Pharmacol.* **1888**, *24*, 247-260.

(2) Scatchard, G.; Scheinberg, I. H.; Armstrong, S. H., Jr. *J. Am. Chem. Soc.* **1950**, *72*, 535-540.

(3) Scatchard, G.; Wu, Y. V.; Shen, A. L. *J. Am. Chem. Soc.* **1959**, *81*, 6104-6109.

(4) Collins, K. D. *Biophys. Chem.* **2006**, *119*, 271-281.

(5) Collins, K. D. *Biophys. J.* **1997**, *72*, 65-76.

(6) Robinson, R. A.; Harned, H. S. *Chem. Rev.* **1941**, *28*, 419-476.

(7) Lund, M.; Jungwirth, P. *J. Phys. Condens. Matter* **2008**, *20*, 494218/494211-494218/494214.

(8) Lund, M.; Vrbka, L.; Jungwirth, P. *J. Am. Chem. Soc.* **2008**, *130*, 11582-11583.

(9) Vondrasek, J.; Mason, P. E.; Heyda, J.; Collins, K. D.; Jungwirth, P. *J. Phys. Chem. B* **2009**, *113*, 9041-9045.

(10) Mason, P. E.; Heyda, J.; Fischer, H. E.; Jungwirth, P. *J. Phys. Chem. B* **2010**, *114*, 13853-13860.

(11) Heyda, J.; Hrobárik, T. s.; Jungwirth, P. *J. Phys. Chem. A* **2008**, *113*, 1969-1975.

(12) Vrbka, L.; Jungwirth, P.; Bauduin, P.; Touraud, D.; Kunz, W. *J. Phys. Chem. B* **2006**, *110*, 7036-7043.

(13) Vlachy, N.; Jagoda-Cwiklik, B.; Vacha, R.; Touraud, D.; Jungwirth, P.; Kunz, W. *Adv. Colloid Interface Sci.* **2009**, *146*, 42-47.

(14) Meyer, D. E.; Chilkoti, A. *Biomacromolecules* **2002**, *3*, 357-367.

(15) McPherson, D. T.; Xu, J.; Urry, D. W. *Protein Expression Purif.* **1996**, *7*, 51-57.

(16) Cho, Y.; Sagle, L. B.; Iimura, S.; Zhang, Y.; Kherb, J.; Chilkoti, A.; Scholtz, J. M.; Cremer, P. S. *J. Am. Chem. Soc.* **2009**, *131*, 15188-15193.

(17) Cho, Y.; Zhang, Y.; Christensen, T.; Sagle, L. B.; Chilkoti, A.; Cremer, P. S. *J. Phys. Chem. B* **2008**, *112*, 13765-13771.

(18) Zhang, Y.; Trabbic-Carlson, K.; Albertorio, F.; Chilkoti, A.; Cremer, P. S. *Biomacromolecules* **2006**, *7*, 2192-2199.

(19) Floss, D. M.; Schallau, K.; Rose-John, S.; Conrad, U.; Scheller, J. *Trends Biotechnol.* **2010**, *28*, 37-45.

(20) Dreher, M. R.; Simnick, A. J.; Fischer, K.; Smith, R. J.; Patel, A.; Schmidt, M.; Chilkoti, A. *J. Am. Chem. Soc.* **2007**, *130*, 687-694.

(21) McDaniel, J. R.; Callahan, D. J.; Chilkoti, A. *Adv. Drug Deliver. Rev.* **2010**, *62*, 1456-1467.

(22) PhaseBio. Core Technology. http://www.phasebio.com/content.php?page=22 (accessed Jan 25, 2012)

(23) Apel, P. Y.; Korchev, Y. E.; Siwy, Z.; Spohr, R.; Yoshida, M. *Nucl. Instrum. Methods Phys. Res., Sect. B* **2001**, *184*, 337-346.

(24) Sexton, L. T.; Horne, L. P.; Martin, C. R. *Mol. BioSyst.* **2007**, *3*, 667-685.

(25) Siwy, Z.; Apel, P.; Baur, D.; Dobrev, D. D.; Korchev, Y. E.; Neumann, R.; Spohr, R.; Trautmann, C.; Voss, K.-O. *Surf. Sci.* **2003**, *532-535*, 1061-1066.

(26) Woermann, D. *Nucl. Instrum. Methods Phys. Res., Sect. B* **2002**, *194*, 458-462.

(27) Siwy, Z.; Fulinski, A. *Am. J. Phys.* **2004**, *72*, 567-574.

(28) Woermann, D. *Phys. Chem. Chem. Phys.* **2003**, *5*, 1853-1858.

(29) Howorka, S.; Siwy, Z. In *Handbook of Single Molecule Biophysics*; Hinterdorfer, P., Oijen, A. v., Eds.; Springer Science&Business Media: New York, 2009, p 293-339.

(30) Wanunu, M.; Meller, A. *Nano Lett.* **2007**, *7*, 1580-1585.

(31) Ali, M.; Yameen, B.; Cervera, J.; Ramirez, P.; Neumann, R.; Ensinger, W.; Knoll, W.; Azzaroni, O. *J. Am. Chem. Soc.* **2010**, *132*, 8338-8348.

(32) He, Y.; Gillespie, D.; Boda, D.; Vlassiouk, I.; Eisenberg, R. S.; Siwy, Z. S. *J. Am. Chem. Soc.* **2009**, *131*, 5194-5202.

(33) Woermann, D. *Phys. Chem. Chem. Phys.* **2004**, *6*, 3130-3132.

(34) Wei, C.; Bard, A. J.; Feldberg, S. W. *Anal. Chem.* **1997**, *69*, 4627-4633.

(35) Siwy, Z.; Kosinacuteska, I. D.; Fulinacuteski, A.; Martin, C. R. *Phys. Rev. Lett.* **2005**, *94*, 048102.

(36) Chen, W.; McCarthy, T. J. *Macromolecules* **1997**, *30*, 78-86.

(37) Ladam, G.; Schaaf, P.; Cuisinier, F. J. G.; Decher, G.; Voegel, J.-C. *Langmuir* **2001**, *17*, 878-882.

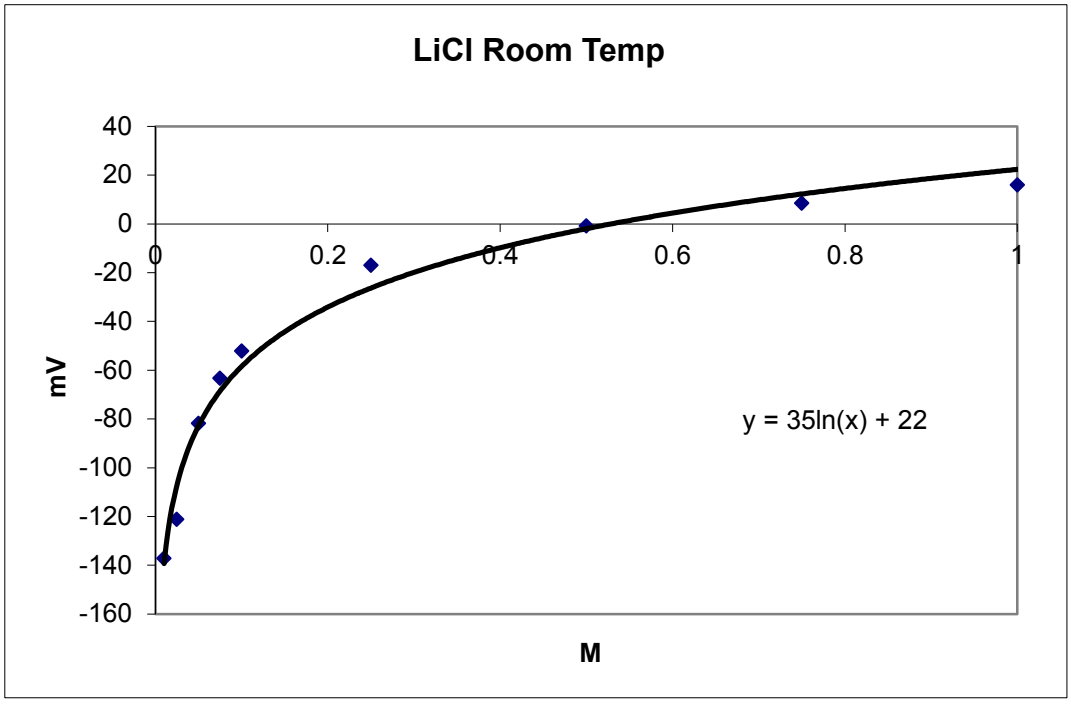
(38) Narayanan, J.; Xiong, J.-Y.; Liu, X.-Y. *J. Phys.: Conf. Ser.* **2006**, *28*, 83-86.

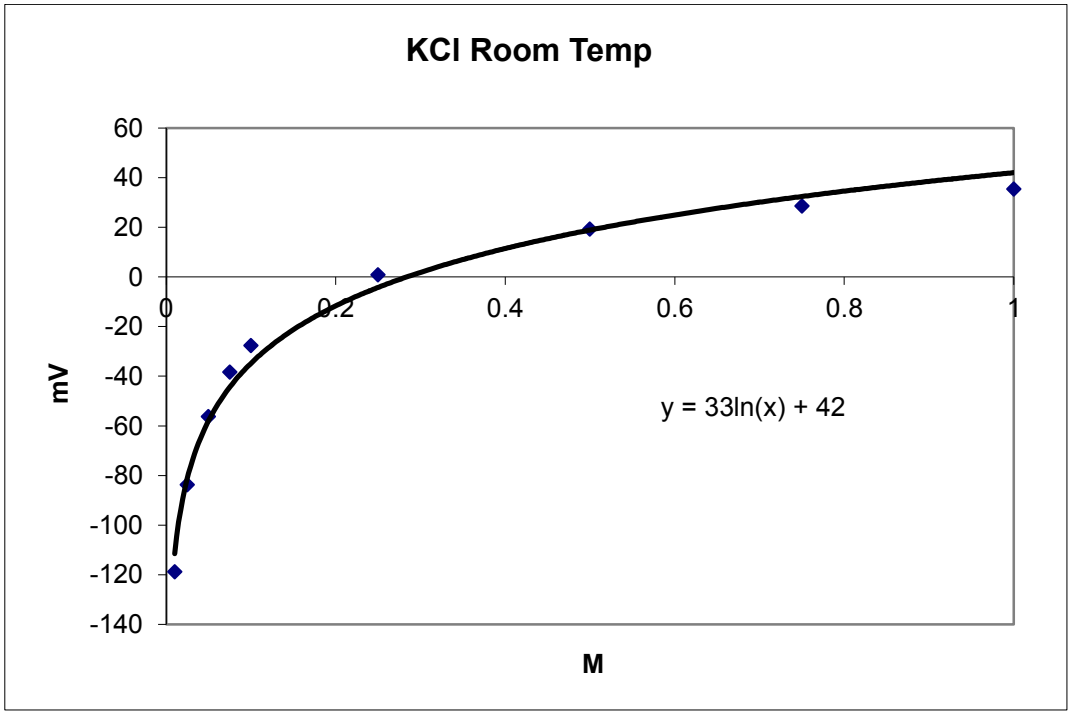
(39) Scopes, R. K. *Protein Purification: Principles and Practice*; 3rd ed.; Springer: New York, 1993.

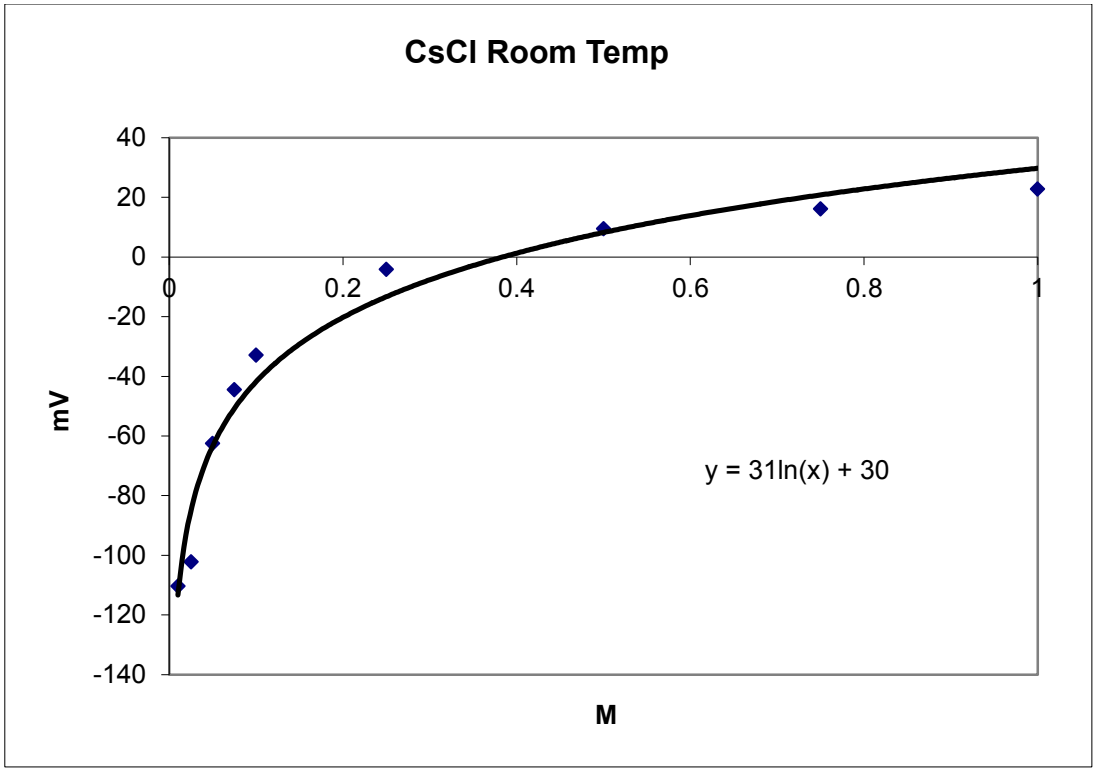
(40) Zhang, B.; Galusha, J.; Shiozawa, P. G.; Wang, G.; Bergren, A. J.; Jones, R. M.; White, R. J.; Ervin, E. N.; Cauley, C. C.; White, H. S. *Anal. Chem.* **2007**, *79*, 4778-4787.

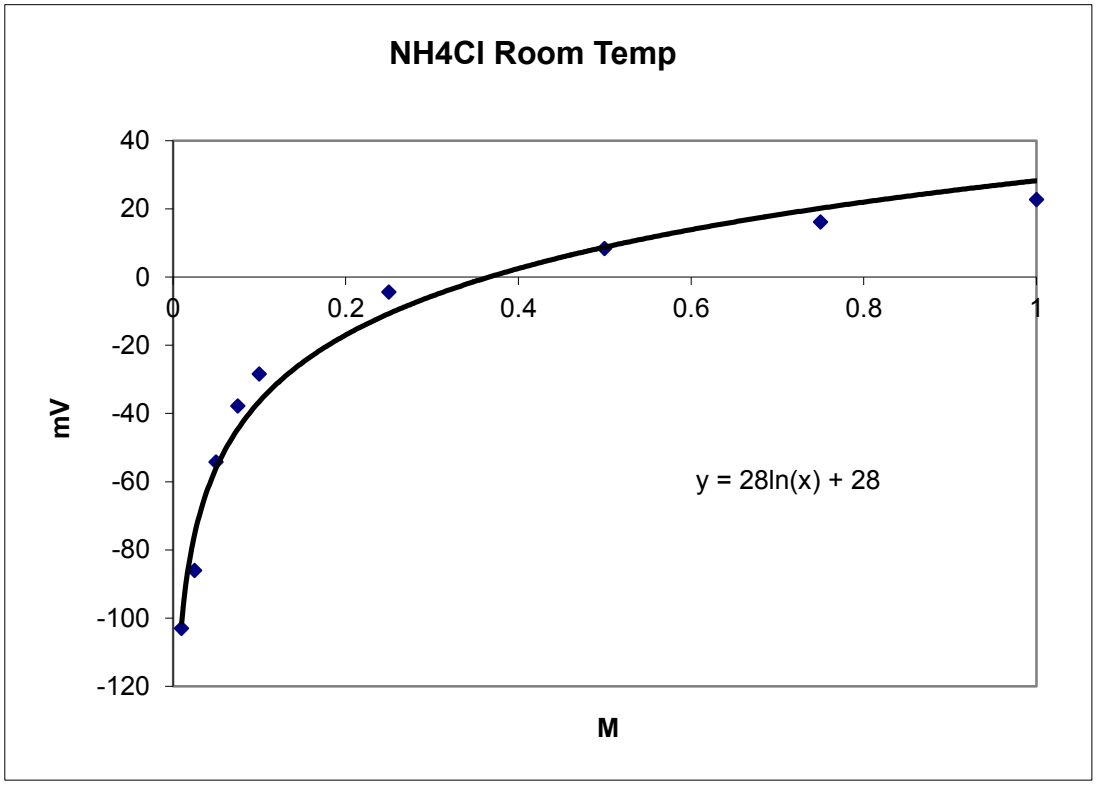
APPENDIX

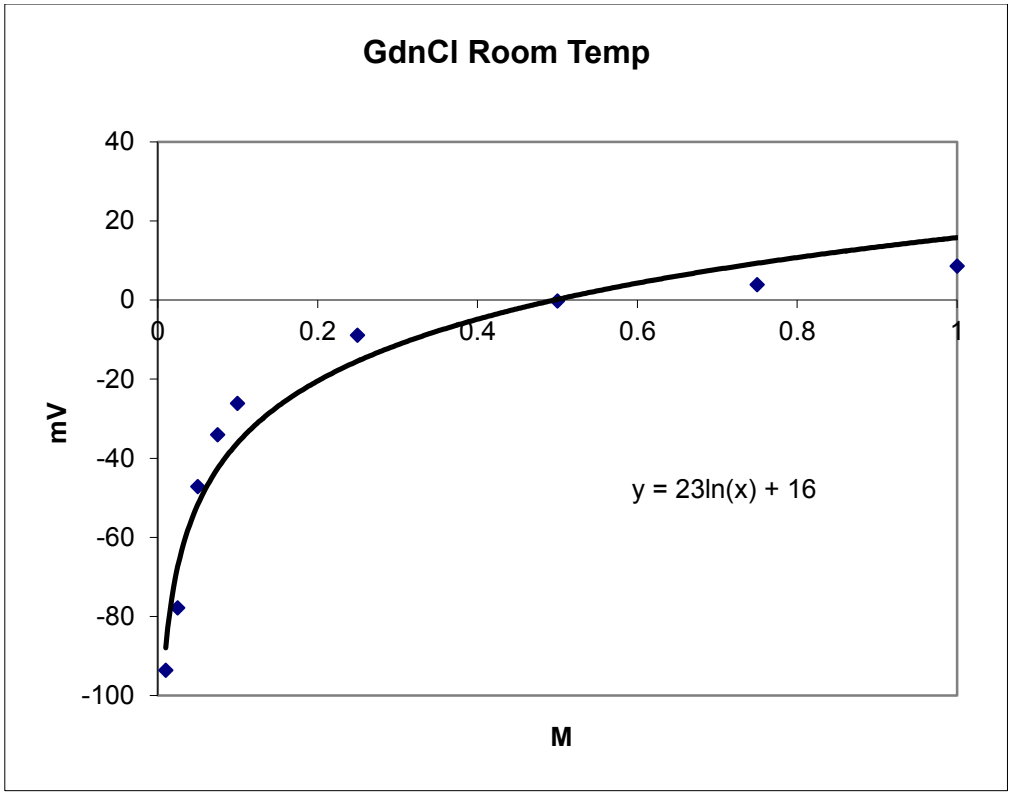
FIGURES

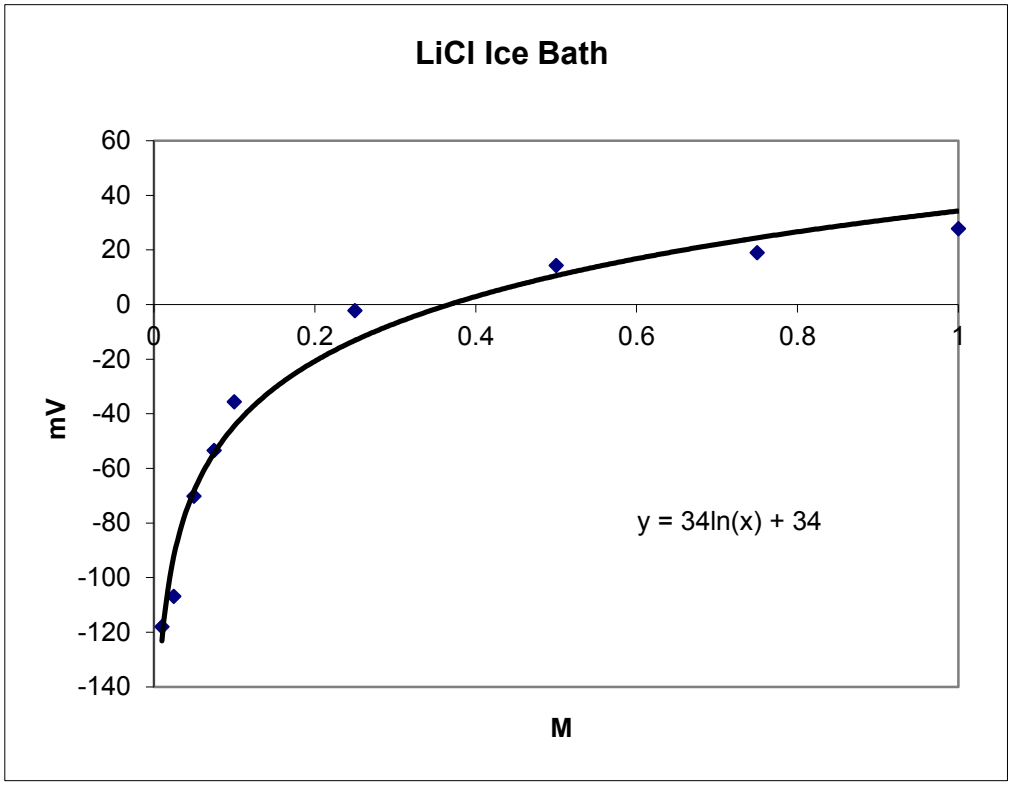


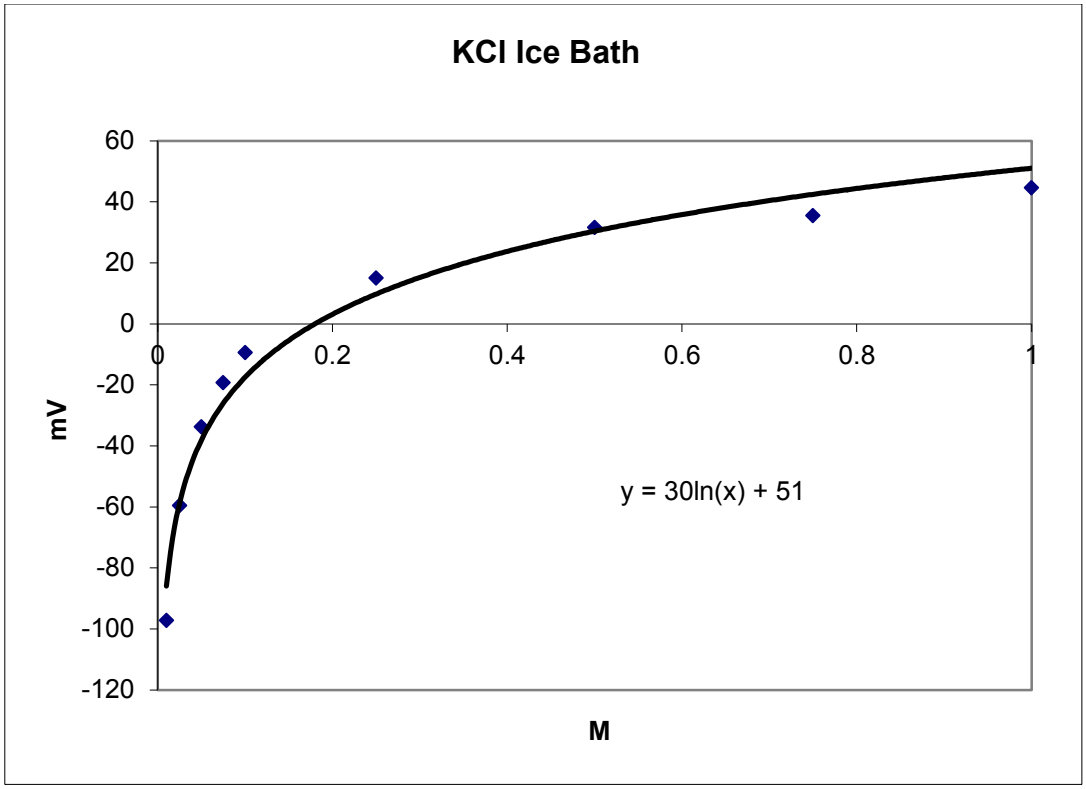


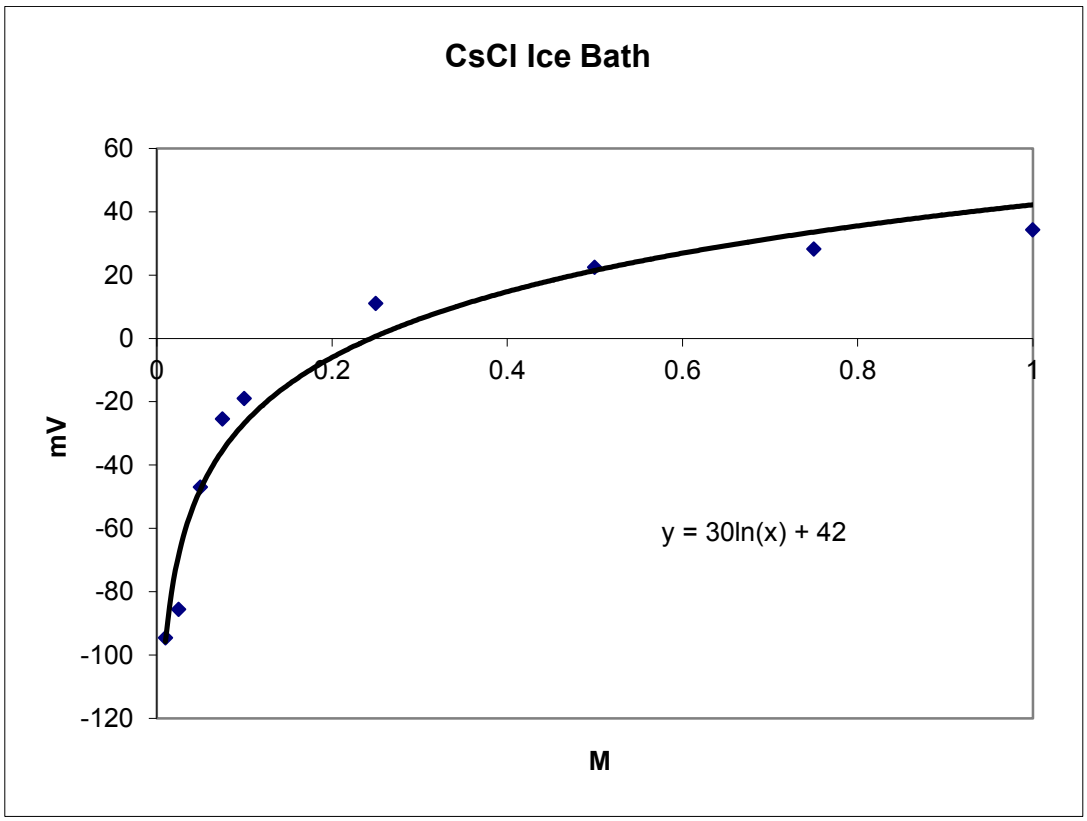

CsCl Room Temp
40
20
0
-20
-40
-60
-80
-100
-120
-140
0
0.2
0.4
0.6
0.8
1
mV
M
y = 31ln(x) + 30

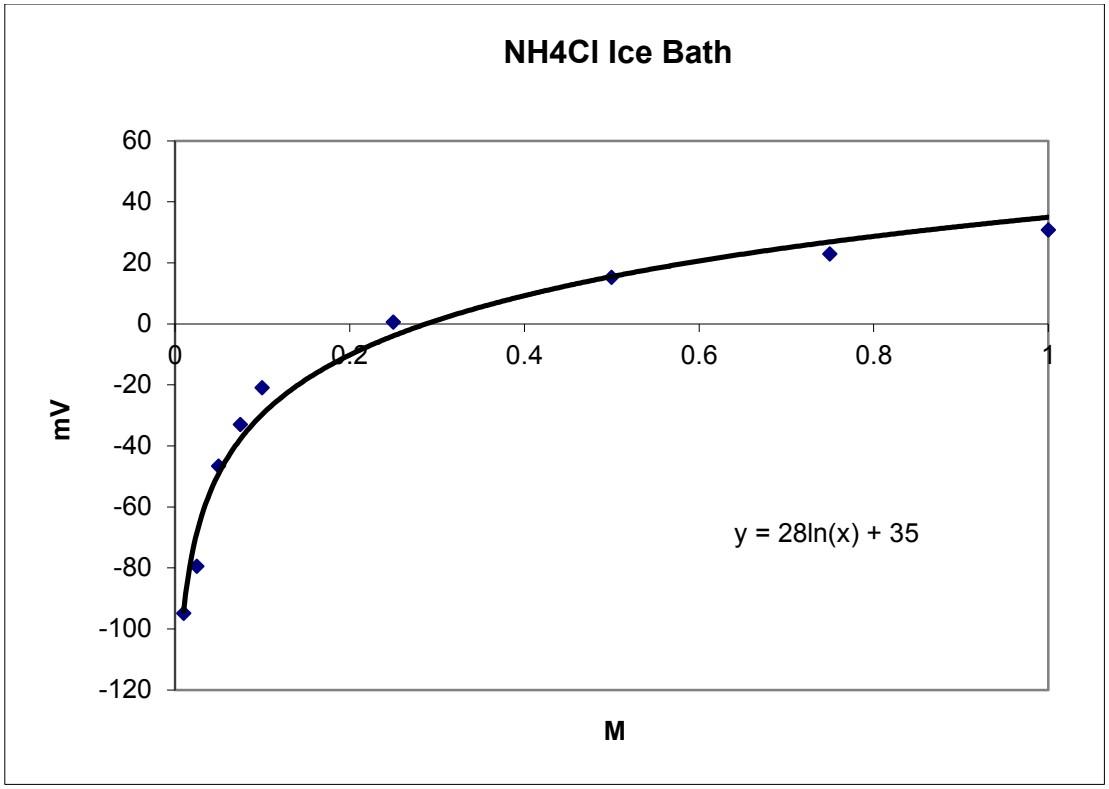

NH4Cl Room Temp
40
20
0
-20
-40
-60
-80
-100
-120
0
0.2
0.4
0.6
0.8
1
mV
M
y = 28ln(x) + 28

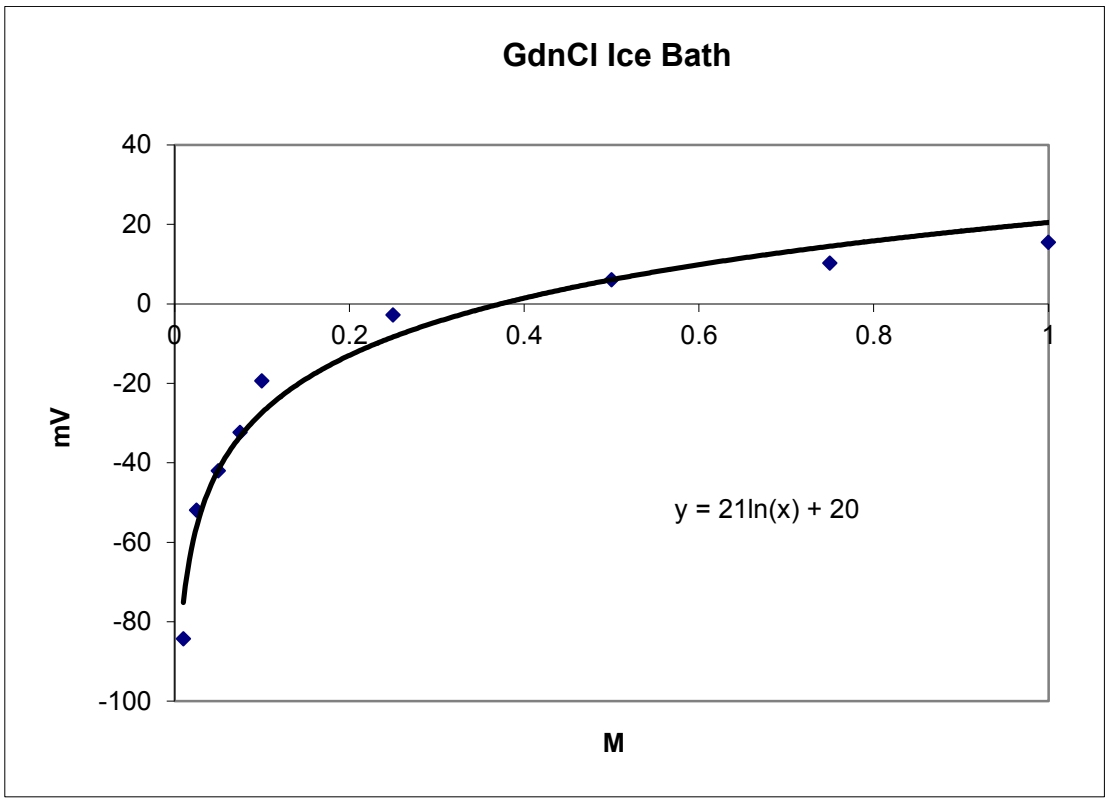

GdnCl Room Temp
40
20
0
-20
-40
-60
-80
-100
0
0.2
0.4
0.6
0.8
1
mV
M
y = 23ln(x) + 16


LiCl Ice Bath
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
0
0.2
0.4
0.6
0.8
1
mV
M
y = 34ln(x) + 34


KCl Ice Bath
60
40
20
0
-20
-40
-60
-80
-100
-120
0
0.2
0.4
0.6
0.8
1
mV
y = 30ln(x) + 51
M


CsCl Ice Bath
60
40
20
0
-20
-40
-60
-80
-100
-120
0
0.2
0.4
0.6
0.8
1
mV
y = 30ln(x) + 42
M


NH4Cl Ice Bath
60
40
20
0
-20
-40
-60
-80
-100
-120
0
0.2
0.4
0.6
0.8
1
mV
M
y = 28ln(x) + 35


GdnCl Ice Bath
40
20
0
-20
-40
-60
-80
-100
0
0.2
0.4
0.6
0.8
1
mV
M
y = 21ln(x) + 20

VITA

Name:	Sean Blount Bard
Address:	Department of Chemistry, Texas A&M University
	PO Box 30012, College Station, TX 77842-3012
Email address:	sbard@terrabon.com
Education:	B.S. Chemistry, Rice University, 2006